

HISTOPATHOLOGIC ANALYSES
OF SCORBUTIC GUINEA PIGS TREATED WITH
SPECIFIC ADRENO-CORTICO-ACTIVE COMPOUNDS

by

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INTRODUCTION

Extracts from the cortex of the adrenal gland were first prepared in 1930 by Hartman and his co-workers (Turner, 73). They prepared an adrenal cortical extract termed "cortin" which is now designated as the unfractionated active ingredients of the adrenal cortex. After this date, more than thirty different steroid compounds have been isolated from the adrenal cortex. Two important components of these known adreno-cortical hormones are 11-dehydro-17-hydroxycorticosterone (cortisone) and desoxycorticosterone, which are necessary for the continued life of the animal.

There are various principles necessary for the production of cortisone in the animal body and at the present time these constituents are indistinct. Apparently one of the important factors is adrenocorticotrophic hormone (ACTH) of the anterior pituitary. The anterior pituitary adjusts its output of ACTH to meet the physiologic requirements of the organism for adreno-cortical hormones. Ascorbic acid is another factor that is thought to be necessary for the secretion of adreno-cortical hormones although the function of this vitamin is not clear.

The experiment was conducted primarily to determine whether ACTH or desoxycorticosterone had an action similar to that of cortisone in preventing arthritic lesions in scorbutic guinea pigs and to compare clinical symptoms, analysis of blood, growth response, feed consumption, adrenal weights, post mortem lesions,

and adrenal ascorbic acid among groups injected with these hormones and ascorbic acid. It would also be an indication as to whether ascorbic acid was necessary for the production of cortisone. Theoretically, if ascorbic acid was not required for the production of cortisone, then the results of guinea pigs given ACTH and the guinea pigs receiving cortisone would be similar.

REVIEW OF LITERATURE

Cortisone

Discovery. The word "cortisone" is a name introduced by Dr. E. C. Kendall of the Mayo Foundation to identify a compound which has previously been referred to as 17-hydroxy-11-dehydrocorticosterone or as "Kendall's compound E" (35).

Cortisone was isolated in crystalline form from extracts of beef adrenal glands in 1935 (21). The isolation and characterization of its general physical and chemical properties were accomplished independently by Mason et al. (49), and by Wintersteiner and Pfiffner (74). A partial synthesis of cortisone from desoxycholic acid has been realized by Sarrett (66).

Relatively large doses of cortisone are required to maintain life in adrenalectomized animals (3),(35). When the compound was first isolated, it was found to be biologically active in the muscle work test of Ingle (35). Larger amounts of cortisone were required for maintaining life than of 11-desoxycorticosterone

acetate (35), (44). It is of special significance that cortisone and other 11-oxygenated compounds are relatively weak in life maintenance activity as compared to 11-desoxycorticosterone but that cortisone is more potent than 11-desoxycorticosterone in sustaining the ability of the adrenalectomized animal to resist stress (34), (35).

Secretion. In considering the secretory mechanism of the adrenal cortex there is one basic factor that cannot be over-emphasized. It is that an increased rate of secretion of at least the 11-oxy-adreno-cortical steroids is only possible following a preliminary release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary (7), (36), (46), (47). The anterior pituitary adjusts its output of ACTH to meet the physiologic requirements of the organism for adreno-cortical hormones (36). This was shown by the removal of one adrenal gland which was followed by compensatory hypertrophy of the remaining adrenal cortex, but this did not occur in the absence of the anterior pituitary. Also, any type of stress to which laboratory animals were subjected caused adreno-cortical hypertrophy. This response was abolished by removal of the anterior pituitary (54), (73).

Haynes et al. (23) studied the effect of ACTH on adreno-cortical slices under conventional incubation conditions. Their data indicated that the output of cortical steroids by slices of adrenal cortex was substantially enhanced by the action of ACTH and that in perfused adrenal glands ACTH accelerated the synthesis of corticosterone hormones and increased the rate of their release

from the adrenal gland.

There is abundant evidence that the anterior pituitary secretes more ACTH into the blood during adreno-cortical insufficiency and suppresses its output of ACTH in the presence of an excess of adreno-cortical hormones (18), (36). The reason for increased amounts of cortical hormones required during stress is that it may be necessary to attain a higher concentration of the cortical hormones in the body fluids during stress, or stress may cause an increase in the utilization of cortical hormone so that a compensatory increase in secretory activity is required to keep a uniform level of hormones in the blood. If a higher concentration of cortical hormones in the body fluids is required during stress, it seems unlikely that this could be achieved if the anterior pituitary is sensitive only to the concentration of cortical hormones per se. It would be more logical to assume that the anterior pituitary is sensitive to some metabolic consequences of cortical hormone action (36).

The rapidity with which the anterior pituitary responds to stress and its anatomic relationship to the central nervous system suggests the possibility that there may be nervous control over the release of ACTH. However, there is general agreement that the infundibulum can be sectioned without preventing the release of ACTH during stress (36).

The humoral theory was the most widely accepted as described by Ingle (36) and by Long (46). In order that this theory may have any weight it is necessary to suppose that all conditions associated with increased secretion of ACTH are first preceded by

an increased utilization of adreno-cortical hormones by the cells of the body, which is followed by a fall in the blood level of adreno-cortical hormones. This in turn stimulates the release of additional ACTH tending to restore the blood level of adreno-cortical hormone to normal (46).

Cronheim et al. (7), injected salicylates into rats and it caused a depletion of adrenal ascorbic acid. The depletion was proportionate to the amount of salicylates used. Hypophysectomy completely abolished the effect of salicylates, thus indicating importance of the anterior pituitary and specifically ACTH in the function of the adrenal cortex. Also removal of the pituitary did not prevent the response of this gland to ACTH.

Methods of Detecting Secretions of the Adrenal Cortex. A variety of methods have been used to detect an increased secretory rate of the adrenal cortical steroids. These include the direct or indirect measurement by chemical or biological methods of the content of the adreno-cortical hormones in the blood in the adrenal vein; the detection of adreno-cortical hormones in lipid extracts of the urine, either by chemical or biological techniques; the fall in adrenal ascorbic acid or cholesterol, which is a specific response of the gland to an increased blood level of ACTH; the fall in the number of circulating eosinophils or lymphocytes, which appears to be a specific response to an increased blood level of adreno-cortical hormones (47).

Ascorbic Acid

Activity in the Adrenal Gland. Increased adreno-cortical activity is associated with a reduction in the ascorbic acid content of the adrenal gland (1), (7), (11), (12), (20), (30), (35), (36), (46), (47), (57). The quantitative relationship between the dose of exogenous ACTH and the extent of fall in adrenal ascorbic acid provides a sensitive test for the bio-assay of ACTH and for measuring rapid changes in adrenal cortical activity during stress.

Ascorbic acid was known to be related intimately to the functional activity of the adrenal cortex, although the exact biochemical mechanisms were still obscure. Evidence had been presented indicating that ascorbic acid was necessary for production of adreno-cortical hormones (54). If stimuli were applied to cause increased production of adreno-cortical hormones, the ascorbic acid concentration in this gland dropped quickly (29). If these stimuli were continued over a sufficient period of time, the adrenal gland enlarged and the ascorbic acid concentration was increased. Some evidence has been presented suggesting that ascorbic acid may be synthesized in the adrenal gland (54).

The fact that this vitamin prevents adrenal enlargement under these conditions might mean that in some manner it can substitute for cortical hormones. This is strengthened, superficially at least, by the fact that if sufficient cortical extract is given to animals under stress, adrenal enlargement will be suppressed (54). Enlargement of the adrenal gland may be a

response enabling the gland to produce more vitamin C. Then there is the possibility that ascorbic acid is used up in the production of adreno-cortical hormones (54).

Nadel and Schneider (52) conducted an experiment which showed that normal male guinea pigs excrete relatively large amounts of adreno-cortical hormones in the urine, and that severely scorbutic animals excrete significantly greater amounts. The enhanced excretion of adreno-cortical hormones in the scorbutic state is of interest. Scurvy probably represents a severe stress situation because the guinea pig elicits increased adrenal activity (57). The increased activity is manifest in a gland in which the ascorbic acid content is at very low levels. While the evidence presented is indirect, it strongly suggests that in the guinea pig, ascorbic acid is not necessary for the elaboration of adreno-cortical hormones.

It may be that the biochemical mechanisms necessary for the production of adreno-cortical hormones simply crowd out ascorbic acid until adaptation has occurred at which time the ascorbic acid concentration returns to a new normal level in proportion to the heightened metabolic activity of the tissue. This assumption would not explain why ascorbic acid prevents adrenal enlargement (54).

An experiment was conducted (53) in which guinea pigs were fed a diet deficient in ascorbic acid, and the content of this vitamin and of the cortical hormones in the adrenal cortex was determined. During this period the ascorbic acid concentration in the adrenal cortex fell from 100 mg. per cent to below 5 mg.

per cent, whereas the cortical hormone (determined by its effect on the pigment cells of the scale of the fish Cyprinus carpio) fell to almost one-third of its original value. If ascorbic acid was injected, a rapid increase of this vitamin as well as of the adreno-cortical hormone occurred. The concentration of both substances reached the values found before the animals were placed on the scorbutic diet. Gairdner (Nutrition Review, 53) concludes, therefore, that the synthesis of the adreno-cortical hormone depends upon the presence of ascorbic acid in the adrenals.

Another experiment was conducted injecting ACTH intraperitoneally, 4 mg. per 100 grams of body weight, in male rats (53). The adrenals were removed at various periods after the injection of ACTH and analyzed for cholesterol and ascorbic acid. The hormone was found to produce a rapid decrease in ascorbic acid concentration of the adrenal tissues. Twenty minutes after treatment with ACTH the ascorbic acid content of the adrenal glands had been reduced one-third and after 1 hour it reached its maximum decrease, 40 per cent of its original value.

It has been postulated that vitamin C functions as a co-enzyme in an oxidation-reduction system which produces the oxy-type of adrenal hormones (56), (69), (71). One of the reasons for this deduction is that many lines of evidence have suggested that ascorbic acid is involved in the biological oxidation of tyrosine (8), (56), (69). Studies with rat liver homogenate preparations and acetone powder extracts have indicated that the effect of ascorbic acid in the conversion of tyrosine to acetoacetic acid is primarily upon the first oxidative step of this

metabolic pathway, the oxidation of *p*-hydroxyphenylphruvic acid. Recently it has been proposed that ascorbic acid is a co-factor for the enzyme catalyzing this oxidative step (56). Sealock and Goodland (69) have shown that liver slices from scorbutic guinea pigs were unable to oxidize tyrosine, as normal slices were able to do, unless the crystalline vitamin C was added. In vitro addition of crystalline synthetic ascorbic acid to scorbutic liver slices made them indistinguishable from normal slices in their ability to oxidize tyrosine as measured by oxygen consumption. Studies with guinea pigs (12) indicate that the main difficulty of vitamin C deficient tissues is an inability to oxidize the side chain of tyrosine rather than a failure to oxidize the benzene ring or conjugate the phenolic groups.

One method of ascorbic acid acting as a co-enzyme could be in oxidizing desoxycorticosterone. It seems most probable that ascorbic acid does oxidize this hormone (20). A method of testing if ascorbic acid oxidizes desoxycorticosterone when injected in combination is to try to obtain the same clinical effect by substituting another oxidizing agent for the ascorbic acid. Hallberg (20) used methylene blue and deducted that if the same substance is formed when desoxycorticosterone methylene blue are injected as with desoxycorticosterone and ascorbic acid, the results would suggest that the substance formed is an oxidation product of desoxycorticosterone. The results were similar with the exception that the effects with desoxycorticosterone injected with methylene blue were of shorter duration because methylene blue has less oxidizing ability than ascorbic acid (20), (55), (56).

Ascorbic acid crystals are stable in air for an indefinite period of time and in solution the vitamin is very easily oxidized. Its instability increases with an increasing rise of pH. Mild oxidation converts l-ascorbic acid to dehydroascorbic acid (3). It is upon the basis of this property that most analyses for ascorbic acid are made.

Chemical and Physical Properties. According to Hawk et al. (22) ascorbic acid crystallizes into white, colorless, odorless crystals having a melting point of 190 degrees to 192 degrees C. The vitamin is soluble in water and most organic solvents. The l-isomer is physiologically inactive. Ascorbic acid owes its acidic properties to ionization of the enol ($\text{HO}-\text{C}=\text{C}$) group on the third carbon atom and not to the carboxyl group which is tied up in a lactone form. It is a comparatively strong acid, 0.5 per cent solution having a pH of 3. It is precipitated by the lead ion at pH 7.6 and the salt may be redissolved in mineral acids at pH 2.

The oxidation product of l-ascorbic acid is dehydro-ascorbic acid and above pH 5, the lactone ring is split yielding diketogulonic acid, which is not biologically active. Diketogulonic acid readily undergoes further oxidation to oxalic acid. Destruction of the vitamin is catalyzed by light and reacts with niacin and other pyridine and quinoline compounds. Ascorbic acid is also readily oxidized to dehydroascorbic acid in plant tissues by ascorbic acid oxidase (2).

Assay. There are numerous methods available in the literature for the quantitative determination of ascorbic acid. Roe

and Keuther's (63), (64) method for the assay of ascorbic acid was based on the reaction of dehydroascorbic acid with 2,4-dinitrophenylhydrazine to form an osazone which, on treatment with sulfuric acid, resulted in a colored dehydration product. This product absorbed maximally at 500-550 and 350-380 Angstrom's unit (0.1 millimicron) in a spectrophotometer. The method of Roe and Keuther (63), (64) was a determination similar to that of Hochberg et al. (27). According to Roe and Keuther (63), (64) and Pijoan and Gerjovich (59) this was an excellent method for the determination of the vitamin C content of blood and urine. But, Pijoan and Gerjovich (59) concluded that the method was not desirable for the assay of ascorbic acid in other tissues and foodstuffs because of a possible loss of lactone structure and the formation of diketogulonic acid. They pointed out that 2,4-dinitrophenylhydrazine will react with diketogulonic acid, possibly phenylpyruvic acid, and other alpha-keto acids. The original method of Roe and Keuther (63), (64) has been improved to include the determination of diketo-l-gulonic acid by Roe et al. (65) and is a very satisfactory method for the determination of ascorbic acid in tissues. The necessity and practical value of an accurate and rapid method of assay for ascorbic acid and its oxidation products is indicated in nutritional diseases and deficiencies as well as in certain microbiological diseases (39), (61).

Scurvy (scorbutus) is caused by a deficiency of vitamin C in the body (3). Vitamin C is synthesized by all animals with the exception of man, monkey, and guinea pig. The main clinical symptoms and lesions of scurvy are great weakness and emaciation

usually within the second week, petichial hemorrhages of the mucous membranes, swollen and painful joints due to subperiosteal or muscular hemorrhage, rough hair coat, and death usually at the end of the third week (3), (10).

Histopathology

Deficiency of Vitamin C. The principle morphological effects of vitamin C deficiency were found in mesenchymal tissues. Intercellular substances such as collagen, osteoid, and dentine fail to be deposited in the normal fashion by their respective cells, fibroblasts, osteoblasts, and odontoblasts (12). In scurvy the small blood vessels became impaired from the lack of intercellular cementing substance and ruptured easily (3), (12), (60). Injury of the small vessels were modified by the amount of stress, protected vessels rarely ruptured (10). The osteoblasts in long bones reverted to simple connective tissue cells and there was the failure for them to form osteoid tissue. A wide zone of calcified, but unossified matrix develops just below the actively growing cartilaginous plate, termed the scorbutic lattice. There was a weakening of the costo-chondral junctions and of the epiphysis of bones. Pathological fractures, separation of the periosteum at the site of muscle insertion, and hemorrhage beneath the periosteum were common (3), (12). Because the pathologic aspect of experimental scurvy was the result of retardation of the deposition of intercellular substances which accompanied growth, the severity of the lesions was influenced

by the general nutritional state of the animal and was expressive of the growth which occurred while the animal was fed the vitamin C deficient diet. Therefore, those guinea pigs which best maintained their weight during the experiment should show the most severe, scorbutic lesions.

In scorbutic guinea pigs osteoblasts lost their round or oval shape and became fusiform. The osteoblasts migrated away from the trabeculae and were indistinguishable from fibroblasts. The proliferative zone of cartilage ceased to grow and it was slowly resorbed, becoming slender up to its end where it suddenly broadened into a thin junctional zone which was usually concave (10). Adjoining this atrophic concave cartilage, was a zone of fibrous tissue in which lie irregular calcified and acellular fragments of pre-existing trabeculae, called "Trummerfeld zone" or region of complete disintegration. Associated with this fibrous tissue were hemorrhagic and irregular masses of acidophilic material, thought to be bone. This material stained brilliant red. Beneath the Trummerfeld zone was an area where there were no hematopoietic cells and which was composed of connective tissue cells, this area is the so called "Gerustmark." (12).

The data presented by Wolbach (77) showed that the clinical evidence of scurvy appeared after an average lapse of 12.2 days. His studies provided proof that in scurvy there was a failure of formation of the matrix of cartilage comparable to that of the matrices of bone and connective tissue. He studied the costochondral junction and the femoral tibial joints. The least adequately described effect of scurvy in guinea pigs was that of

epiphyseal cartilage (76), (77). Follis et al. (14) found that the cells of the epiphyseal and costal cartilage continue to proliferate and mature and that the matrix formation of bone ceases in young guinea pigs. Matrix formation of bone, connective tissue cells, and cartilage cells did not mature until ascorbic acid was given. He also found distortion of cartilage cell columns. The area of abnormal cartilage was bounded on the epiphyseal side by the layer of reserve or indifferent cartilage and on the diaphyseal side by a zone of calcified cartilagenous matrix deposited before the deficiency develops.

In scurvy animals mitosis often seemed arrested at the metaphase stage although there was an extraordinary increase of mitosis in the fascicular zone of the adrenal gland. The cells of the fascicular zone were swollen, pale, finely vacuolated, and contained but little lipid. Glomerular zone of the adrenal gland became atrophic and the cells were small and without lipid droplets (77).

Other pathologic conditions of scurvy were pronounced necrosis of the muscle tissue with a tendency to calcify and there was the presence of giant cells which represent abortive attempts at regeneration (10). The only myopathy reported by Wolbach and Maddock (77) was an atrophy of skeletal muscles. Other pathologic changes associated with scurvy were fatty degeneration of the myocardium, suspended estrus cycle if the deficiency was severe, atrophy of the adrenal glands, spleen, salivary glands, kidneys, and the liver, hemorrhage and erosions of the stomach and intestinal tract (10). Eisenstein (11) reported that the pathologic

criterion of vitamin C-deficiency was an adrenal hypertrophy. Hypertrophy of the adrenal gland in scurvy had been substantiated by various authors (25), (30), (67), (77).

In working with guinea pigs, Herrick et al. (25) found that all animals on a vitamin C-free diet exhibited pain about the joints after 11 days and all were dead after 19 days. Survival time on the vitamin C-free diet ranged from 12 to 19 days. Histological preparations of ribs and heel joints of all animals were made and studied. Little bone tissue other than some of the perichondral type remained in scorbutic animals. The adrenal glands were also studied from the scurvy animals and the cortical cells were enlarged and there were degenerative changes with vacuolation and disintegration of these cells. There was an increase in the lipid content in the zona fasciculata. They also found many mitotic figures at the junction of the zona glomerulosa and zona fasciculata of the adrenal glands.

Cortisone Injections. The attention of laboratory and clinical investigators to the biologic effects of cortisone has centered on only a few tissues. There was no reason to assume that the effects of cortisone and similar compounds were limited to any specific organ or tissue or that any tissue was unresponsive to them. There were probably gradients in the sensitivity of tissues to the adreno-cortical hormones and some tissues may be protected from the metabolic actions of these hormones to a greater extent than others by as yet unknown homeostatic mechanisms (35).

Following the preparation of biologically active extracts of

beef adrenal glands 20 years ago, the erroneous concept developed that hormones of the adrenal cortex do not damage the organism when administered in "high doses". Adrenal cortex extracts have seldom been found toxic because it is difficult to administer doses which exceed the normal secretory activity of the adrenal cortices of either animals or humans. In the case of the crystalline compounds of the adreno-cortex, it is easily possible to administer doses which cause damage to tissues (32), (35). Ingle (35) compared female and male rats in respect to their resistance to large amounts of cortin (adreno-cortical extract) for a period of one week. The female rat was more resistant to over-doses of cortin than was the male rat. Although it was possible to cause pathologic changes by the administration of very large amounts of cortisone to animals and damage was more likely to occur under certain abnormal conditions, it seemed probable that physiologic amounts and doses equivalent to therapeutic doses in man did not cause any irreversible pathologic changes (33).

As a result of careful studies of conditions which favor remission of rheumatoid arthritis, such as pregnancy, hepatitis with jaundice, surgery, and starvation, it was postulated by Hench and Kendall of the Mayo Clinic that the secretory activity of the adrenal cortices must be stimulated in each of these conditions and this secretion might play a role in causing the remissions. When sufficient amounts of cortisone became available in the fall of 1948, studies of its effects in arthritis were undertaken by investigators. Their announcement of the favorable effect of cortisone and of adrenocorticotrophic hormone on rheuma-

toid arthritis, in April of 1949, further emphasized the potential reversibility of this disease and led to the testing of these and chemically related compounds in other collagen diseases (33).

Follis (13) injected 40 or 50 mg. of cortisone per kilogram of body weight into rats. He noted a narrowing of the epiphyseal cartilage due to reduction in number of hypertrophic cells and also there was retardation in the proliferation of undifferentiated cells. More prominent was the presence of an increased amount of calcified cartilagenous matrix encased in bone beneath the epiphyseal cartilage. This zone continually increased in width so that after three weeks there was a broad zone of dense interlacing trabeculae each with a central core of cartilagenous matrix encased in bone. There did not appear to be an excess number of osteoblasts nor were osteoblasts present in any conspicuous form.

There would appear to be two possible ways to explain the pathogenesis of the changes noted by Follis (13). There may be an increased osteoblastic activity or decreased osteolytic activity or a combination of both. It does not appear however, to be an increase in number of osteoblasts.

ACTH Injections. In the studies by Hyman et al. (30) the effects of cortisone and ACTH on scurvy guinea pigs are similar. This finding lends support to the view that deficiency of ascorbic acid does not appreciably interfere with the production of adrenocortical steroids. Cortisone was given ten days after the beginning of the scorbutic diet. They lived from 19 to 34 days, an average of 26.5 days. The onset of clinical scurvey was approxi-

mately one week later in the cortisone treated group than in the scorbutic controls. There was appreciably less hemorrhage in the cortisone group than was found in the untreated groups, although bloody diarrhea, hematuria, and hind leg paralysis appeared in most of them. ACTH was also given ten days after the inauguration of the scorbutic diet. The average length of life was 29.8 days. The pathologic conditions found in the group treated with ACTH were pulmonary edema, bloody diarrhea, hematuria, and hind leg paralysis. The pathologic conditions of the ACTH group developed approximately eight days later than in the scorbutic control group. Hemorrhagic manifestations compared favorably to those occurring in the cortisone treated group and less than in the untreated scorbutic controls and the ACTH treated group was too low for measurement, below 2.5 micrograms per 100 mg. of fresh adrenal gland. The adrenal glands of the cortisone treated group contained 2.5 micrograms of ascorbic acid per 100 mg. of fresh adrenal gland and the adrenal gland of the normal control animals contained 81.3 micrograms of ascorbic acid per 100 mg. of fresh adrenal gland.

The histopathologic changes in the scorbutic control guinea pigs were an increased cell size, enlarged vesicular nuclei, and increased cytoplasmic granules of the adrenal gland. The group of guinea pigs injected with ACTH showed the same pathologic lesions but with a marked increase in mitotic figures. The adrenal gland of the cortisone treated group contained small cells with few granules and pyknotic nuclei being typical of involution (30).

Desoxycorticosterone Injections. Schaffenburg et al. (67)

presented evidence that indicates an antagonistic action between desoxycorticosterone and cortisone. He used four groups of guinea pigs, one group of guinea pigs was a normal control and the other three groups were on a vitamin C-deficient diet. One of the three groups was injected with desoxycorticosterone, a second group was given cortisone, and the third group was used as a scorbutic control. Only the guinea pigs receiving desoxycorticosterone and the scorbutic controls showed symptoms of scurvy. The cortisone group did not show scorbutic symptoms and they did not vary in behavior from the normal controls. The scorbutic symptoms appeared first and most severe in the group treated with desoxycorticosterone. On the tenth day the desoxycorticosterone group manifested decreased muscular activity and apathy. The guinea pigs' fur was dull and dirty. By the fifteenth day most of this group were prostrate and dyspneic. Two guinea pigs in the scorbutic control group died on the fifteenth and seventeenth days and the rest of the group deteriorated rapidly. There was no obvious difference in joint swelling between the group receiving desoxycorticosterone and the negative scorbutic control other than it appeared first in the desoxycorticosterone group. Cortisone prevented joint swelling in all guinea pigs except one. Hemorrhage occurred in all groups except the positive control. The hemorrhage was more severe in the guinea pigs receiving desoxycorticosterone than in the other groups of guinea pigs and there was less hemorrhage in the cortisone treated pigs than the other groups with the exception of the

positive controls (67). Most of the hemorrhage was subcutaneous with some occurring intramuscularly and in the joints. Apparently desoxycorticosterone aggravates the scorbutic condition while cortisone inhibits the scorbutic symptoms.

There was evidence that hyaluronidases may play a role in the pathogenesis of rheumatoid arthritis by attacking the synovial structures (35). It has been shown that cortisone has an antagonistic action on the effect of hyaluronidase (6), (38), (62). Layton (45) theorized that cortisone may exert its action through the inhibition of the synthesis of certain mucopolysaccharides by connective tissue cells. It has also been postulated that the action of cortisone on arthritis or any pathologic condition may be due to a suppression of the symptoms or the surface indications (5), (38).

A prevailing idea is that cortisone has influence on mesenchymal tissues in relation to the maintenance or integrity of intercellular substances, of which collagen is the chief or an important constituent. Wolbach and Maddock (77) attempted to substantiate this hypothesis. Cortisone was injected intraperitoneally in 12, 12.5, 15, and 25 mg. doses in guinea pigs weighing 159-190 grams on the eighteenth to the twenty-second day of scurvy. Six hours after the first injection the guinea pigs were etherized lightly, bled from the heart, and destroyed. Giving cortisone on the eighteenth to the twenty-second day of scurvy did not indicate that it prolonged the life of the guinea pigs. Gross lesions as indicated by hemorrhage were not altered by cortisone injections when these animals were compared with the

scorbutic controls.

Molomut et al. (50) studied the effects of cortisone on the spleen. He injected 1 mg. of cortisone intraperitoneally twice a day. Sections of the spleens revealed smaller Malphigian bodies and fewer mononuclear and lymphocytic cells in the pulp. There were no observable abnormal cells or degeneration.

Significant gains in the weight of adrenal glands can be detected 12 hours after the beginning of stress. This hypertrophy can be duplicated with large injections of ACTH in hypophysectomized rats (31) and scorbutic guinea pigs (11), (30). This adrenal hypertrophy was not due to intake of water entirely because there was a gain in dry tissue. Increase in adrenal weight was probably due to a functional adaptation to the increased requirements of the body for cortin. Adrenal hypertrophy did not occur in hypophysectomized rats in response to stress (11), (30). Small doses of ACTH had no effect on the adrenal weight (9). Adrenal atrophy occurred with injections of cortisone (37), (67), (68). Schaffenburg et al. (67) reported an increase in adrenal weight when injecting desoxycorticosterone.

The data presented by Hyman et al. (30) showed a failure for scorbutic guinea pigs to gain weight from the beginning of the experiment. Injections of cortisone in scorbutic guinea pigs indicated the weight of the guinea pigs to level off for about 14 days after the scorbutic diet was started and then dropped rapidly until death. Cortisone given ten days after the scorbutic diet caused a relatively steep fall in weight after seven days of therapy and continued until death (30). Scorbutic

guinea pigs treated with ACTH showed a modest weight loss after initiation of a vitamin C-free diet followed by a plateau or slight rise in weight and this weight gain was followed by a progressively rapid fall until death (11), (30).

Forty or 50 mg. of cortisone per kilogram of body weight were administered to rats (13). After an initial gain for one or two days the growth curves flattened out and remained so during a three to four week interval. Amounts of cortisone greater than 50 mg. per kilogram led to prompt plateauing of the growth curve followed by a gradual loss of weight. Amounts less than 40 mg. per kilogram of body weight showed no effect on growth. Schaffenburg (67) injected desoxycorticosterone in scorbutic guinea pigs and they began losing weight from the beginning of the experiment. The desoxycorticosterone treated guinea pigs had a weight loss similar to the scorbutic controls.

Guinea Pig Blood

Erythrocyte Counts, Hematocrit, and Sedimentation Rates.

The erythrocyte of the guinea pig is a non-nucleated biconcave disc which appears orange in Wright-stained preparations. The average of the reported values for the number of erythrocytes in the blood of the adult guinea pig, regardless of sex, is 5.64 million per c.mm. (16). King and Lucas reported that the range of erythrocytes is 4.70 million to 5.25 million per c.mm. of blood. The volume of packed red cells was found by Goodman, Geiger, and Klumpp to average 42.0 per cent, with a range of 37

to 47 per cent (16). The average sedimentation rate of erythrocytes for 90 guinea pigs was found to be 1.06 mm. at the end of 1 hour. The sedimentation rate had increased to 4.0 mm. at the end of 6 hours (16). They found that, although the fourth hour was the best for interpretation, the maximum sedimentation was sometimes not reached for 41 days. They found, too, that the sedimentation rate was only slightly influenced by the source of the blood, by hemorrhage, diet, sex, pregnancy, jaundice, or repeated bleedings.

Leucocyte and Differential Counts. The average of the reported values for the total number of leucocytes, regardless of sex, was 9,800 per c.mm. of blood with a range, reported by Ber, of 6,000 to 18,000 (16). An average absolute increase of 3,057 cells per c.mm. of blood was found in the leucocyte count of seven normal guinea pigs over a 5 hour period (16). The averages of the reported values (16) for the different types of cells, expressed as the number per 100 leucocytes are as follows:

Neutrophils	40.89 (31-53)
Eosinophils	2.65 (1.6-3.5)
Basophils	0.45 (0.19-0.7)
Lymphocytes	53.81 (39.4-63.6)
Monocytes	2.89 (1.5-3.2)

Anemia of vitamin C deficiency is generally the hypochromic type (44). This anemia may be associated with iron deficiency or as a result of profuse hemorrhage due to the vitamin deficiency itself. Hemorrhage is probably due to vascular weakness since platelet, coagulation, and bleeding time are normal (41),

(60). There are very few changes in the leucocyte picture in vitamin C deficiency.

Muehrcke et al. (51) reported that 4 hours after injections of corticotropin (ACTH) into healthy individuals the blood eosinophil count was found to be reduced. This also developed after treatment with 17-hydroxycorticosterone (compound F), corticosterone (compound B), and cortisone. Best and Samter (4) showed data in which eosinopenia resulted with the injection of ACTH and that the maximal depression occurred in about 4 hours and subsided within 12 to 24 hours.

In the experiment conducted by Gabrilove (15), administration of ACTH caused a decrease in circulating eosinophils. When ACTH was discontinued the eosinophil count rose markedly. In two instances the eosinophil count returned to its original level while 100 mg. of ACTH was being administered daily. Sprague et al. (Ingle, 35) found no significant change in the number of circulating eosinophils after the prolonged administration of cortisone acetate in their series of patients, but they did observe eosinopenia in some patients after short periods of treatment. It may be assumed that the effect of cortisone and of similar compounds upon the blood cells was either lost after a time or was masked by an increase in the rate of formation of these cells (35). Gabrilove (15) showed that the eosinophil count was reduced markedly upon administration of cortisone in two patients.

Data presented by Eisenstein and Shank (11) indicated that the number of circulating eosinophils decreased progressively throughout the experiment in five of eight animals on a vitamin

C-deficient diet. At the beginning the average count of eosinophils was 127 ± 11.3 per mm^3 . When the guinea pigs approached severe scurvy, all the eosinophil counts approached zero. No animal to which ascorbic acid was administered was found to have a progressive decline in the eosinophil counts. Upon the injection of ACTH there was a fall in eosinophils followed by a progressive increase in the scorbutic guinea pigs. The increase of eosinophils following ACTH injections was thought to be due to adrenal insufficiency, therefore to determine if the adrenal gland could still respond to ACTH, large doses were administered. In each animal that received large doses of ACTH there was a decrease of 50 per cent or more of eosinophils.

Speirs and Myer (Ingle, 35) found that eosinophils of the adrenalectomized mouse were very sensitive to the 11-oxy-steroids and were less sensitive to 11-desoxycorticosterone. As little as 3 micrograms of cortisone produced a 96 per cent decrease in eosinophils in 4 hours. Doses smaller than 1 microgram caused some degree of eosinopenia. Malkiel (48) showed that cortisone and also ACTH caused a decrease in relative mononuclear and an increase in polymorphonuclear leucocyte count.

Hills *et al.* (26) injected 25 mg. of ACTH intramuscularly in a human. After an initial short latent period there ensued a fall in lymphocytes and eosinophils and a rise in neutrophils, commonly resulting in an increase of the total leucocyte count. These results were always apparent after 2 hours and maximal at about 4 hours. Leucocytes approached the normal values in the next 4 to 6 hours. There was a neutrophil increase of 104 per

cent, mean lymphocyte decrease of 39 per cent, and eosinophil decrease of 73 per cent upon injection of ACTH. The maximum change of hematocrit was a 2 per cent fall. Dosage over a period of time resulted in a neutrophilia and a reduction of eosinophils. Both of these results were striking as well as sustained throughout the experiment. Lymphocytes were depressed after the first day, then returned to normal, and later increased beyond normal. After withdrawal of ACTH the eosinophils increased above normal and also the lymphocytes reached their highest level at this time. These changes were also produced by cortisone. They attribute these reactions to adrenal response and it did not occur in its absence.

Hungerford et al. (29) suggested that the eosinopenia activity was not directly correlated with the adrenal ascorbic acid-depleting activity of various ACTH preparations. Normal and hypophysectomized male rats 60 to 80 days of age, were used. Eosinophil counts were made from blood taken from the tail vein of rats. An initial count was taken, followed immediately by intraperitoneal injections of solutions to be tested and a second count was taken 4 hours later. They presented evidence to indicate that there was a lack of correlation between eosinophil activity and adrenal ascorbic acid-depletion activity assayed in normal and hypophysectomized rats. There was an inverse relationship between adrenal ascorbic acid depletion activity and eosinophil activity in the normal rat given ACTH. Some evidence suggested the presence of an eosinophilic component in certain ACTH preparations.

Three main explanations have been advanced to account for the peripheral eosinopenia that follows the administration of adrenal hormones and the application of stress (58). These relate to the inhibition of the production or release of these cells from the bone marrow, redistribution to other organs, e.g. the spleen, and increased destruction. Although chronic treatment with cortisone did eventually induce marrow eosinopenia in the adrenalectomized rat (58), other evidence did not support the contention that the peripheral eosinopenic state caused by adrenal factors was due actually to a disturbance in bone marrow function. With regard to the second possibility, Spain and Thalhimer (Padaver and Gordon, 58) have suggested, on the basis of their data, that migration of eosinophils to the spleen occurred following a single injection of 2.5 mg. of cortisone into mice. It is difficult, however, to reconcile this suggestion with the observations in the present and other reports that the cortisone, epinephrine, or stress-induced peripheral eosinopenia occurs in splenectomized as well as in normal animals (mice, rats, dogs). Absence of splenic arterio-venous differences in eosinophil counts following ACTH or epinephrine in the dog would also militate against this idea (58). It had been suggested (58) that the eosinopenic state may result from actual destruction of eosinophils, but no direct evidence for this possibility, up to now, has been presented. The present experiments provide support for the cytogenic hypothesis that eosinophilic cells undergo destruction in the body fluids. Thus, there is little doubt that degenerating eosinophilic forms are encountered in appreciable

numbers in peritoneal and pleural fluids, and in peripheral blood, especially under circumstances that lead to pronounced eosinopenia in these fluids. Since they are also seen in the body fluids of normal animals, it may be inferred that the cytologic events described above provide a mechanism for the normal disposal of the eosinophilic leucocytes. It would seem possible that, as for the peritoneal eosinophil, the peripheral eosinophils undergo degeneration within the vascular channels and are subsequently ingested, as smaller degenerated bodies, by fixed macrophages lining blood sinusoids within the reticulo-endothelial organs (58).

Also of interest are the data which indicate that a reduction in the concentration of eosinophilic cells following cortisone or epinephrine occurred not only in circulating blood but in peritoneal fluid as well (58). The significant positive correlation between the percentage drop in eosinophilic cell concentrations within the peripheral blood and peritoneal fluid in rats under the various treatments applied in these experiments would tend to suggest that eosinophils are exposed to similar destructive forces in all body fluid compartments.

It has been theorized that the level of circulating lymphocytes is inversely related to the level of cortical secretions (28). Hypophysectomy causes an increase in lymphocytes (28). This increase in lymphocytes becomes apparent within 24 hours and was still observed 3 weeks later. It was also higher than in pair fed animals given the same amount of food as actually consumed by the hypophysectomized animals. There was a moderate

elevation of the total cell count of thoracic lymph but not an increase in lymph flow in adrenalectomized animals (28). There was also an increase in circulating lymphocytes in adrenalectomized animals. A single injection of ACTH two hours later in normal rats caused the total cell content per unit volume of lymph and the lymph flow to be decreased. ACTH exhibited no effect when administered to adrenalectomized rats, as compared with control adrenalectomized rats. With prolonged administration of ACTH in normal rats the lymph flow was significantly reduced on an absolute basis and relative to body weight. The total lymphocyte count was significantly lowered on an absolute basis; however, the cell count was unaltered relative to body weight. There was no significant difference in cell count and lymph flow in hypophysectomized rats compared with control rats. The prolonged injection of ACTH did not decrease the level of lymphocytes to the extent of a single injection. There was no significant alteration in lymph flow and the total cell count when adrenal cortical extract (aqueous), cortisone acetate, or desoxycorticosterone glucoside were injected intraperitoneally.

Garcia et al. (17) demonstrated that there was a low hemoglobin concentration in hypophysectomized animals. This actually reflects a decrease in red blood cell volume. He injected 0.5 mg. of ACTH intraperitoneally twice a day. There was no significant change in total red blood cell volume in normal animals after 34 days of treatment. In hypophysectomized animals ACTH treatment prevented a decrease in total red blood cells.

Data presented by Selye and Carey (70) indicated that rats

treated with desoxycorticosterone showed a small but statistically significant increase in erythrocyte sedimentation rate. When cortisone was administered, the erythrocyte sedimentation rate was extremely low and almost negligible after 1 hour. For determination of erythrocyte sedimentation rate he used the micro-sedimentation technique of Cutler.

MATERIALS AND METHODS

Thirty female guinea pigs weighing 300 to 450 grams were purchased from the Gopher State Caviary, 826 Atlantic Street, St. Paul 6, Minnesota. The guinea pigs were numbered from one to 30 and identified by notching their ears. The animals were then randomized by the method of Snedecor (72) into five separate groups. There were seven guinea pigs in group I, five guinea pigs in group IV, and six guinea pigs in each of groups II, III, and V. The guinea pigs were housed in a room thermostatically controlled at a temperature of 71 to 72 degrees F. Also the animals were retained in steel wire cages and each group was divided into two subgroups and each of these subgroups occupied one cage.

All groups of guinea pigs were given a vitamin C-deficient basal ration (Table I) and with the exception of group I, which was the negative control, each guinea pig in each group was supplemented daily as follows:

Group II	5 mg. of cortisone acetate ¹
Group III	5 Wilson units of ACTH ²
Group IV	5 mg. of desoxycorticosterone acetate ³
Group V	4.3 mg. of ascorbic acid ⁴

The cortisone, ACTH, and desoxycorticosterone were injected subcutaneously in their respective groups at 8 hour intervals and the vitamin C was injected subcutaneously into the guinea pigs of group V once daily. There were 25 mg. of cortisone acetate per cc. and the quantitative amount was 0.07 cc. per injection. The ACTH preparation contained 40 Wilson units per cc. and the amount of each injection was 0.04 to 0.05 cc. There were 5 mg. of desoxycorticosterone per cc. administered in doses of 0.33 cc. per injection. Thirty mg. of vitamin C were diluted with 3.5 cc. of a neutral physiological solution of NaCl. Each 3 cc. of the diluent contained 0.12 cc. of 0.1 N NaOH. Each cc. of this preparation contained 8.57 + mg. of vitamin C and the guinea pigs were given 0.5 cc. per injection as recommended by Ruether et al. (43). The hormones and vitamin C were administered with tuberculin syringes using 25 or 26 gauge hypodermic needles.

The guinea pigs were fed in round crock containers. An upright wire mesh was placed in the center of the crocks to prevent the guinea pigs sitting in their feed. These feed containers

1. Cortone acetate (11-dehydro-17-hydroxycorticosterone-21-acetate), Merck and Co.

2. Corticotropin, Wilson.

3. Cortate, Schering Corp.

Table 1. Vitamin C-free basal ration for guinea pigs.

Ingredient	%	Pounds	Grams
Ground oats	64.5	32.25	14,596
Soybean meal	10	5.0	2,260
Heated, dried skimmed milk	5	2.5	1,135
Brewer's yeast, dried	5	2.5	1,135
Casein, vitamin-free	5	2.5	1,135
Corn oil	5	2.5	1,135
Steamed bone meal	1	0.5	228
Salt, iodized	1	0.5	228
Cod liver oil, potency			
Vitamin A 1800 USP units			
Vitamin D 180 USP units	1	0.5	228
Ground limestone	2.5	1.25	567.5
Vitamin B ₁₂ Supplement (Merck's supplement No. 3 contains 12.5 mg/lb.)			36.3
Trace minerals (Calcium Carbo- nate Co. Contains:			
MnSO ₄ 20.0%			
FeSO ₄ 40.0%			
CuSO ₄ 2.0%			
CoSO ₄ 0.933%			
ZnSO ₄ 0.589%			17
Total	100	50	22,700.8

were never excessively filled in an attempt to reduce the amount of feed wasted. They were replenished whenever necessary to provide a constant supply of feed to the guinea pigs. The feed was weighed into large glass containers and at four day intervals the amount of feed left in the feed crocks was weighed and added to the weight of the feed left in the glass container. This total weight of feed was subtracted from the initial weight of feed and this number was used as the total amount of feed consumed in each specific group. The guinea pigs were also weighed at four day intervals.

The guinea pigs were examined daily for clinical symptoms of scurvy or any gross pathological changes. They were examined closely for joint swellings, pain upon palpation, hair coat, position of animal at rest, reaction to agitation, and general appearance.

The guinea pigs were restrained on a bleeding board and 2 cc. of blood were obtained by cardiac puncture on designated days throughout the experiment. On the first day of the experiment, the first guinea pig of each group was bled, the second day of the experiment the second guinea pig of each group was bled, then on eight and nine day intervals from the last day of bleeding, blood was obtained from the next successive guinea pigs of each respective group. This method was continued until the termination of the experiment. Guinea pigs that did not succumb during the experiment were bled and killed with chloroform. Two successive bleedings of the same guinea pig were avoided and a total of 34 blood samples were studied.

Each blood sample was placed in a test tube which contained 2.4 mg. of dry ammonium oxalate and 1.6 mg. of potassium oxalate. This anti-coagulant was worked out by Heller and Paul (24) and is described by Wintrobe (75). A solution of the anticoagulant was prepared by dissolving 1.2 gm. of ammonium oxalate and 0.8 gm. of potassium oxalate in 100 ml. of neutral distilled water. Five-tenths cc. of this solution was measured into a series of test tubes and evaporated to dryness.

The erythrocyte and leucocyte counts were made by the standard methods (41). For the determination of total erythrocytes, the blood was drawn to the 0.5 mark of the red blood cell diluting pipet. Toisson's diluting fluid was then drawn to the 101 mark of the pipet, thus making a dilution of 1:200. The blood and diluting fluid were shaken for one minute by an electrical pipet shaker. In counting erythrocytes, 80 of the smallest squares of a bright-line improved Neubauer hemacytometer were counted. This was done by using the 4 mm. objective on a microscope and counting the erythrocytes in the four corner squares and one center square of the finely ruled central area of the counting chamber. The five counts had to fall within a range determined by the square root of the average as described below. The number of cells found in 80 small squares, divided by 80, determined the average number of erythrocytes per small square or in 0.00025 c.mm. The number of cells counted in 0.00025 c.mm. was multiplied by 4000 to determine the number of cells in 1 c.mm. of diluted blood, and by 200 to determine the number in 1 c.mm. of undiluted blood. Therefore in routine work the number of

cells in 80 small squares were counted and four ciphers added. In counting the erythrocytes both counting chambers were filled from separate pipets containing blood from the same animal. The counts were made on both sides and averaged. If the counts varied too much, as described below, new counts were made by taking new samples of diluted blood from the pipets. To determine if the two counts varied excessively the square root was taken of the average of the two counts. If this number was greater than the difference between the counts and the average, the cells were considered to be from the same population.

Leucocytes were counted in a similar manner as erythrocytes. The blood was drawn to the 0.5 mark of a leucocyte diluting pipet. Diluting fluid was then drawn to the 11 mark of the pipet, thus making a dilution of 1:20. The diluting fluid was made by mixing 3 cc. of glacial acetic acid with 97 cc. of distilled water. This fluid produces complete hemolysis of erythrocytes. The blood and diluting fluid were shaken for one minute by an electrical pipet shaker. The cells were counted in the four corner squares on the improved Neubauer counting chamber. Each of these 4 square millimeter areas was subdivided into 16 squares to facilitate counting. Counts were made by using the 16 mm. objective of a microscope. The total number of leucocytes counted in the four squares were multiplied by 50 to obtain the number in 1 c.mm. of undiluted blood.

A differential leucocyte count was made by staining the blood smear with May-Grunwald and Giemsa combination stain. The staining procedure of the freshly drawn blood is as follows:

May-Grunwald 1 min.

Eosin stain 10 sec.

Giemsa stain 15 min.

The Giemsa stain had to be replenished after eight slides had been stained. Additional smears would not take the basic stain.

In making the differential leucocyte counts, 200 cells were counted by the four-field meander method described by Gradwohl (19). A Marbel's blood-cell calculator was used to record the various cells while making the counts.

The hemoglobin content of the guinea pig blood was determined by the acid hematin method of Cohen and Smith (Hawks, et al. 22). The Coleman Spectrophotometer was used in making this determination. A stock standard acid hematin solution was prepared after the iron content of a sample of guinea pig blood was determined by the method described by Wong (78). The blood was then diluted with 0.1 N HCl in a 100 ml. volumetric flask so that the stock standard acid hematin solution contained 3 per cent hemoglobin. This stock standard will keep three months in a refrigerator according to Hawk et al. (22). From the stock solution, the dilute standard was prepared fresh every week by diluting 5 ml. to 200 ml. in a volumetric flask with 0.1 N HCl. This dilute standard was an acid hematin solution equivalent to 0.075 grams per cent hemoglobin. Calculations for hemoglobin: $\frac{\text{Density of unknown}}{\text{Density of standard}} \times 0.075 \times$

$$\frac{100}{0.06} \times \frac{10.05}{100} = \text{grams of hemoglobin per 100 ml. of blood.}$$

The 0.075 represents the hemoglobin content of the standard, in grams per 100 ml.; the 0.05 is the volume of blood taken, and the 10.05 represents the volume to which the blood was diluted.

The sedimentation rate of the erythrocytes was determined by filling Wintrobe hematocrit tubes with blood. The sedimentation rate was determined at 1, 2, and 4 hour intervals. The tubes were held in a vertical position in a sedimentation rack. The tubes were filled with blood by using Wintrobe filling pipets. At the end of 4 hours the hematocrit tubes were centrifuged for 30 minutes at 3,000 r.p.m. The volume of packed erythrocytes expressed in per cent was recorded.

All guinea pigs that died during the experiment or were killed at the end of the experiment were studied for gross pathological lesions. They were observed for hemorrhage, especially subcutaneous, hemorrhage in the joint capsules and around the joints, edema, and lesions on the viscera and organs of the body. Also, the size of the adrenal gland was noted.

At the termination of the experiment two guinea pigs from each group were bled from the heart and then killed with chloroform. The animals were photographed before euthanasia. They were then opened with a subcutaneous, ventral mid-line incision through the thoracic and abdominal regions, the skin was reflected and the carcass photographed. Tissues were removed from the animals for histo-pathological studies. The tissues included were the heart, spleen, liver, kidney, skeletal muscle, tibial-femoral joint, and adrenal glands. The tissues were fixed in Zenker's solution and prepared for microscopic examination. The tissues were stained

with hematoxylin and eosin stain.

Two additional guinea pigs from each group were destroyed toward the termination of the experiment when it appeared they would succumb in a few hours. Immediately after euthanasia the adrenal glands, kidneys, and portions of the liver were removed and analysed for vitamin C. The adrenal glands were carefully weighed and recorded for comparison of size among groups. The procedure for vitamin C determination is described by Roe and Knether (63), (64), (65). Five-tenths to 2 grams of tissue were weighed and analysed. The tissue was ground with mortar and pestle with about 10 ml. of 6 per cent trichloroacetic acid, using acid washed white sand to aid in the homogenization if necessary. The homogenized supernatant was decanted into a 25 ml. graduate or volumetric flask, and the residue was ground with two additional 5 to 7 ml. portions of 6 per cent trichloroacetic acid, transfer the supernatants to the same graduate or volumetric flask. Finally the solution was made up to volume, transferred to a centrifuge tube, and centrifuged. The supernatant fluid was decanted into a 50 ml. Erlermeyer flask or test tube, one-half teaspoonful (0.75 g.) of acid washed Norit was added, stoppered, shaken vigorously, and filtered through a fluted filter paper.

For color development and reading of ascorbic acid values, 4 ml. of the Norite filtrate and 1 drop of 10 per cent thiourea solution was measured into each of two matched colorimeter tubes, labeled sample and blank. To the sample tube 1 ml. of the 2 per cent dinitro-phenylhydrazine reagent was added and kept in a water bath at 37 degrees F. for 3 hours. Then both tubes were

placed in a bath of ice water. While in the ice water bath 5 ml. of 85 per cent sulfuric acid was added to each tube gradually, drop wise, in the course of 1 minute with constant shaking. Finally to the blank tube, 1 ml. of the 2 per cent dinitro-phenylhydrazine reagent was added. It was shaken well and both tubes allowed to stand at room temperature for 30 minutes.

With the 540 millimicron filter in a photoelectric colorimeter, the blank tube was placed in the instrument and the galvanometer set at 100. Then the reading of the sample tubes was taken. From Table 2 the millimicrons of vitamin C from the galvanometer reading was obtained. The colorimetric measurement gave the micrograms of ascorbic acid in 4 ml. of Norit filtrate. For tissues the micrograms of ascorbic acid in 4 ml. of Norit filtrate was multiplied by $25/4$. The results are expressed as micrograms of ascorbic acid per gram of tissue.

Table 2. Calculation chart for use with Photoelectric colorimeter. Mg. of Ascorbic Acid against Galvanometer reading.*

G	GC	G	GC	G	GC	G	GC
100	0.0	80	7.5	60	17.7	40	34.7
99	0.3	79	7.9	59	18.4	39	35.9
98	0.6	78	8.3	58	19.1	38	37.1
97	0.9	77	8.7	57	19.8	37	38.3
96	1.2	76	9.1	56	20.5	36	39.6
95	1.5	75	9.5	55	21.2	35	41.0
94	1.9	74	10.0	54	21.9	34	42.4
93	2.3	73	10.5	53	22.6	33	43.9
92	2.7	72	11.0	52	23.3	32	44.5
91	3.1	71	11.5	51	24.1	31	47.2
90	3.5	70	12.0	50	24.9	30	48.9
89	3.9	69	12.5	49	25.7	29	50.7
88	4.3	68	13.0	48	25.5	28	52.6
87	4.7	67	13.0	47	27.4	27	54.7
86	5.1	66	14.1	46	28.4	26	56.9
85	5.5	65	14.7	45	29.4	25	59.3
84	5.9	64	15.3	44	30.4		
83	6.3	63	15.9	43	31.4		
82	6.7	62	16.5	42	32.5		
81	7.1	61	17.1	41	33.6		

* Roe, J.H. and C. A. Keuther. Biol. Chem. 147:399-407. 1943.

RESULTS AND DISCUSSION

Clinical Symptoms

All groups, with the exception of the group receiving ascorbic acid, showed varied degrees of scorbutic symptoms. On the eighth day group IV receiving desoxycorticosterone showed the first evidence of scurvy, they were despondent and moved very little. By the thirteenth day, the joints of the guinea pigs in Group IV were swollen and they exhibited signs of pain. The hair coat of guinea pigs in group IV was dull and dirty. After eleven days the guinea pigs receiving ACTH, group III, were less active than they had been previously, and on the thirteenth day symptoms appeared that were similar to those present in group IV, but not as severe. The hair coat of guinea pigs in group III was dull and dirty. One guinea pig receiving ACTH was afflicted with swollen joints, and all the animals of this group showed signs of pain. Severe scorbutic symptoms were shown by all guinea pigs in group I by the thirteenth day. All the guinea pigs had swollen and painful joints and showed dull and dirty hair coats. The animals in group II evidenced only slight pain and no articular enlargement during the same period. The hair coat of the guinea pigs in group II was in good condition and they were active and alert in their movements.

Erythrocyte Counts

The guinea pigs in group IV receiving desoxycorticosterone showed a consistent decline in the total circulating erythrocytes. In the other groups the differences in the erythrocyte counts were not statistically significant as shown in Table III. All of the counts fell in the range reported by King and Lucas (40) for average erythrocyte counts with the exceptions of groups III and IV. A consistent decline in erythrocytes occurred in group III until after the tenth day and then on the eighteenth day there was a slight rise in total erythrocytes.

Total and Differential Leucocyte Counts

There were not significant differences statistically in total leucocyte counts among the five groups (Table 4). The guinea pigs receiving cortisone, ACTH, and desoxycorticosterone showed a higher average neutrophil count (Table 5) for the length of the experiment than did the guinea pigs in Group V. There was also a lower lymphocyte count in the groups receiving injections of cortisone, ACTH, and desoxycorticosterone (Table 6). The lymphocyte counts among the five groups were not of statistical significance. A more consistent decline in lymphocytes was found in the guinea pigs receiving injections of cortisone than was found in the guinea pigs in the other groups. In all groups there appeared to be a decrease in neutrophils (Table 5) as the experiment progressed, but it did not follow a definite pattern. Guinea pigs in groups

Table 3. Total and average number of erythrocytes per c.mm. of blood, in millions, for individual guinea pigs.

Group Number	Days of Experiment					Avg.	Standard Deviation
	0	1	9	10	18		
I	5.530	5.080	6.750	5.270	4.980*	5.522	$\sqrt{\quad}$.718
II	4.850	5.370	5.480	4.940	5.027**	5.133	$\sqrt{\quad}$.276
III	5.360	4.970	4.650	3.395	4.760***	4.6270	$\sqrt{\quad}$.740
IV	5.400	4.790	4.105	3.975	3.552*	4.364	$\sqrt{\quad}$.731
V	6.210	5.225	5.210	5.190	5.108**	5.388	$\sqrt{\quad}$.459
Avg.	5.470	5.085	4.190	4.540	4.685	5.006	

* average of two different guinea pigs

** average of three different guinea pigs

*** average of four different guinea pigs

Table 4. Average number of leucocytes per c.mm. of blood for the entire experiment.

Group	Average number of Leucocytes	Standard Deviation	Range
I	8,865	$\sqrt{\quad}$ 4,635	3,550-15,600
II	9,181	$\sqrt{\quad}$ 5,970	4,475-19,400
III	11,785	$\sqrt{\quad}$ 5,267	7,824-20,200
IV	11,895	$\sqrt{\quad}$ 3,937	5,575-16,200
V	10,608	$\sqrt{\quad}$ 4,364	6,940-16,450

Table 5. Average number of mature neutrophils per c.mm. of blood of each group for the entire experiment.

Group	Average number of mature Neutrophils:	Standard Deviation	Range
I	3,703	2,230	994-6,048
II	4,672	4,122	2,241-11,834
III	5,632	4,757	2,223-13,938
IV	5,201	2,736	1,505-8,607
V	3,388	2,529	1,452-7,220

Table 6. Average number of lymphocytes per c.mm. of blood of each group for the entire experiment.

Group	Average number of Lymphocytes	Standard Deviation	Range
I	4,912	2,704	2,526-9,279
II	4,314	2,026	2,168-7,469
III	5,650	1,364.5	4,410-7,883
IV	5,840	3,628	1,115-8,413
V	6,777	2,221	4,650-9,130

Table 7. Average number of eosinophils per c.mm. of blood of each group for the entire experiment.

Group	Average number of Eosinophils	Standard Deviation	Range
I	56.6	61.1	0-156
II	40	34.6	0-97
III	258.8	226.	0-556
IV	88.6	110.8	0-268
V	108.4	113	0-242

II, III and IV usually had a higher neutrophil count than was found in group V. The average number of eosinophils for the entire experiment was lower in groups I, II, and IV than in group V (Table 7). There was an eosinophilia in group III compared with the normal animals. The increase in eosinophils in group III is in direct contradiction to the results of Hills et al. (26) and Best (4). They reported a decrease in total eosinophils with injections of ACTH into normal animals. This work is in partial agreement with Eisenstein and Shank (11) who reported a decrease in eosinophils upon injection of ACTH into scurvy guinea pigs, followed by a progressive increase. Hungerford, et al. (28) suggested an inverse relationship between adrenal ascorbic acid and eosinophil activity in the normal rat given ACTH. The method of counting eosinophils employed in this experiment was not the method of choice.

There were no statistical differences in basophils (Table 8) and immature neutrophils (Table 9) among the five groups. More basophils were present in the blood of guinea pigs receiving ACTH than in any of the other groups. The guinea pigs receiving injections of desoxycorticosterone and the animals in group I had a high average monocyte count compared to the other three groups (Table 10). However, the counts among individual guinea pigs in each group varied so much that it was not of statistical importance.

Sedimentation Rate and Hemoglobin Values

The group of guinea pigs receiving ACTH showed an increased sedimentation rate at the end of four hours (Table 11). There were no significant differences among the other groups. Group IV manifested a consistent decrease during the experiment in packed erythrocytes. There were no statistical differences among groups I, II, III, and V (Table 12). Also, the guinea pigs in group IV manifested the lowest amount of hemoglobin but it was not of statistical significance (Table 13).

Growth Data

Guinea pigs in group I and group II manifested a sharp decline in weight from the first day of the experiment (Table 14). Group III maintained its weight for the first eight days and then ensued a sharp decline. The guinea pigs in group IV gained weight for the first 12 days of the experiment, followed by a decrease in weight. The positive control animals gained weight steadily throughout the experiment. The feed consumption of the guinea pigs in each group was directly related to growth (Table 15). Group I consumed the least amount of food and conversely group V consumed the most. Guinea pigs in group IV ate more feed than guinea pigs in group III and group III consumed more feed than the guinea pigs in group II.

Table 8. Average number of basophils per c.mm. of blood of each group for the entire experiment.

Group	Average number of basophils	Standard Deviation	Range
I	25.2	34.9	0- 70
II	11.8	16.8	0- 36
III	162.8	206.8	0-513
IV	0	0	0
V	0	0	0

Table 9. Average number of immature neutrophils per c.mm. of blood of each group for the entire experiment.

Group	Average number of immature Neutrophils	Standard Deviation	Range
I	20.2	33.8	0- 78
II	26.8	38.8	0- 91
III	25	43.7	0-101
IV	18.8	42.	0- 94
V	0	0	0

Table 10. Average number of monocytes per c.mm. of blood of each group for the entire experiment.

Group	Average number of monocytes	Standard Deviation	Range
I	135.6	127.7	0-257
II	46.8	50.1	0-107
III	53.2	80.4	0-181
IV	136.4	151.1	56-405
V	31.8	39.7	0- 79

Table 11. Average sedimentation rate in mm. of red blood cells of each group for the entire experiment.

Group :	Hours :	Avg. Sedimentation Rate :	Standard Deviation :	Range
I	1	0.5	± 0.67	0-1.5
	4	1.2	± 2.68	0-6
II	1	0.5	± 0.14	0-1
	4	0.54	± 0.65	0-1.5
III	1	0.94	± 1.72	0-4
	4	2.92	± 4.83	0-11.5
IV	1	0.46	± 0.25	0-1.8
	4	1.56	± 1.5	0-4
V	1	0	± 0	0
	4	0.16	± 0.36	0-0.8

Table 12. Average percent of packed erythrocytes of each group for the extent of the experiment.

Group :	Average Hematocrit :	Standard Deviation :	Range
I	38.9	± 3.7	33.5-44
II	40	± 4.2	36 -47
III	42.9	± 8.6	31 -54
IV	33.8	$\pm 6.$	29 -44
V	43.4	± 1.4	41.5-45

Table 13. Average grams of hemoglobin per 100 ml. of blood of each group for the entire experiment.

Group	Avg. hemoglobin content per 100 ml	Standard Deviation	range
I	18.10	2.73	14.44-22.09
II	17.37	1.9	15.36-18.76
III	17.76	4.67	11.36-21.04
IV	15.12	4.67	10.28-22.8
V	20.13	1.5	18.90-22.43

Table 14. Average total weights of guinea pigs of each group, expressed in grams.

Group:	Days of Experiment						Avg.	Std.
Number:	0	4	8	12	16	19		Dev.
I	343.5	354	315	280	235	202	285	56.8
II	372.83	375.1	358.66	316	273.6	238	322	54
III	335	354.5	339.5	327	255	254.3	308	40.4
IV	386	394.6	404.8	406.4	361.6	355.5	370	22
V	366.5	368	386.5	391	402.6	403	386	16
Avg.	360.73	361	361	344	305.5	290.5	334	42.5

Ascorbic Acid Analysis

There was found to be a highly significant difference (1 per cent level) in adrenal ascorbic acid between group V and the other four groups (Table 16). A high adrenal ascorbic acid content was found in the positive control animals and there was a low content of adrenal ascorbic acid in the guinea pigs of the other four groups. The adrenal glands of animals in group I contained the lowest amount of ascorbic acid. The adrenal glands in guinea pigs in group III had slightly more adrenal ascorbic acid than was found in guinea pigs in group I. Animals in groups II and IV had a higher adrenal ascorbic acid content than guinea pigs in group III. Adrenal ascorbic acid in guinea pigs in group IV was slightly higher than in guinea pigs of group II; however, there was not enough difference to be of statistical value.

The liver of guinea pigs in group V contained a high amount of vitamin C (Table 16). The difference between group V and the other four groups was highly significant. There was no significant differences among groups I, II, III, and IV. Animals in group I had the lowest amount of vitamin C in their livers and the liver of guinea pigs in group II contained more vitamin C than those in groups III or IV.

Renal ascorbic acid of guinea pigs in group V was also higher than in kidneys of animals in the other four groups (Table 16). There was very little difference among the first four groups in ascorbic acid content of the kidney.

Table 15. Average grams of feed consumed per guinea pig for the first 16 days of the experiment.

Groups	Days of Experiment				Total	Standard Deviation
	0-4	4-8	8-12	12-16		
I	97.0	52	28	7	184	38.6
II	95.0	63	42	10	210	37.0
III	100.0	73	67	25	270	31.5
IV	108.0	93	72	38	311	30.5
V	119.0	121	105	113	458	26.1
Avg.	100.4	82	54	35.4	256.6	22.5

Table 16. Micrograms of ascorbic acid per gram of tissues (average of two guinea pigs in each group). Determinations were made at the termination of the experiment.

Tissue	Group Number				
	I	II	III	IV	V
Liver	1.8175	8.67	6.638	5.198	61.853
Adrenal	10.231	44.225	11.805	52.31	282.0645
Kidney	5.3953	6.115	3.7193	5.6661	35.9135

Table 17. Adrenal weights of two guinea pigs per group expressed in grams.

Group	Weights		Average
I	0.3	0.46	0.38
II	0.21	0.17	0.19
III	0.54	0.48	0.51
IV	0.46	0.35	0.405
V	0.21	0.31	0.26

Adrenal Weights

The adrenal glands in groups I, III, and IV were larger than the normal controls and the adrenal glands from group II were smaller than the normal controls (Table 17). There was a significant difference between adrenal weights from group II and groups I, III, and IV. The difference in adrenal weights between groups II and III were highly significant and this was the widest range of differences among the five groups. There was also a statistical significant difference in adrenal weights between group III and group V. This hypertrophy of the adrenal glands in guinea pigs in groups I, III, and IV may be due to an attempt by the adrenal gland to secrete sufficient cortisone to meet the requirements of the body. The smaller adrenal glands of guinea pigs injected with cortisone was probably due to the inactivity of the adrenal gland because cortisone was administered in doses sufficient for body requirements.

Autopsy Findings

A detailed post mortem procedure was followed in observing the guinea pigs for pathological changes. The predominate pathology was subcutaneous hemorrhage, swollen and hemorrhagic joints, and skeletal muscle hemorrhage.

With the exception of the guinea pigs in group V, subcutaneous hemorrhages were found in all groups of guinea pigs. The guinea pigs in group IV showed extreme subcutaneous hemorrhage

and the group receiving cortisone manifested slight subcutaneous hemorrhage. In groups I and III subcutaneous hemorrhages were severe, but not as extensive as in group IV.

Swollen and hemorrhagic joints were found in all guinea pigs receiving desoxycorticosterone and in the negative controls (group I). They were also found in one guinea pig in group III. The guinea pigs in groups I and IV showed minute amounts of skeletal muscle hemorrhage. The hemorrhage was most severe in the muscles surrounding the joints. Four guinea pigs in group III manifested minute amounts of skeletal muscle hemorrhage. There were no swollen or hemorrhagic joints in groups II and V.

Histopathology

Bone. In the scorbutic controls, group I, the bone pathology was pronounced (Fig. 1 and 2). There was thickening of the periosteum with increased vascularity. The periosteum was separated from the bone and there was hemorrhage between the periosteum and the perichondral bone. There was a reversion of perichondral bone to an embryonic form of bone. The perichondral was very vascular. The surface of the epiphyseal cartilage appeared irregular, suggesting weakness. In the matrix of the bone of the scorbutic controls there was an atrophic, concave cartilage line. Also there was a wide zone of calcified but unossified cartilagenous plate directly below the cartilage line, "Trummerfeld zone". An extensive regression of bone matrix to an embryonic form of bone was shown in the scorbutic animals and the osteoblasts were reverted to a fusiform fibro-blast like cell.

This embryonic bone stained a light pink and resembled fibrous connective tissue. The Gerustmark zone, migration of bone marrow cells leaving connective tissue elements, was more pronounced in this group than in any of the other groups.

In the group receiving cortisone there was very little pathology of the bone (Fig. 3 and 4). Perichondral bone was normal and there was no subperiosteal hemorrhage. The bone matrix still retained most of its structure. There was the presence of some embryonic bone but it maintained most of the characteristics of mature bone. The surface of the epiphyseal cartilage was irregular and areas showed embryonic type of tissue.

The bones of the scorbutic guinea pigs treated with ACTH showed an atrophic cartilaginous line and a typical Trummerfeld zone beneath it (Fig. 5 and 6). There were subperiosteal hemorrhages and also hemorrhage in the bone matrix. The surface of the epiphyseal cartilage was irregular and had reverted to an embryonic tissue. There was also a reversion of perichondral and matrix bone to an embryonic type of bone. The periosteum was greatly thickened and vascular. The Gerustmark zone was very apparent in group III. The guinea pigs receiving ACTH showed more bone pathology than guinea pigs receiving cortisone .

Group IV manifested a pronounced increased cellular activity of the bone marrow (Fig. 7 and 8). This was evidenced by an increased number of leucocytes. There was an extreme degeneration of the perichondral bone. The periosteum was hypertrophied and vascular. An atrophic cartilaginous line and a distinct Trummerfeld zone was evident and the bone matrix was composed

almost entirely of embryonic bone.

Ovaries. The ovaries from guinea pigs in group I contained no corpus luteum near the surface, indicating no recent ovulation. There were no large follicles present in this group of guinea pigs which presents further evidence of an anovulatory period. In group II there were large follicles containing ova near the surface and there was a large corpus luteum in the center of the ovary. The large follicles and large corpus luteum indicate an ovulatory cycle. There were also large follicles near the surface in the group receiving ACTH, but no corpus luteum was found. In group IV the ovaries from one guinea pig contained no large follicles or corpus luteum. In this same group, the second guinea pig contained two large follicles but no corpus luteum. In group V the ovaries contained large follicles and also large corpora lutea.

Muscle. There was no observable myopathology of skeletal muscle of any of the guinea pigs in the five groups. The cardiac muscle was also normal in all of the observed animals. Macroscopic examination showed skeletal muscle hemorrhage in guinea pigs in groups I, III, and IV. This hemorrhage was largely confined to the areas around the joints. The muscle for microscopic examination was sectioned from the heavy muscles of the thigh.

Liver. The livers of all of the observed guinea pigs were normal.

Spleen. The spleens of the guinea pigs in group IV were composed mostly of leucocytes (Fig. 11). There was a small amount of red pulp. The guinea pigs in the other four groups possessed

normal spleens (Fig. 12).

Kidney. There was no renal pathology in guinea pigs from the five groups.

Adrenal Glands. The adrenal glands from guinea pigs in group I (Fig. 13) showed an increased mitosis arrested at the metaphase stage. The cells of the fascicular zone were swollen and the cytoplasm appeared granular. There was a decrease in vacuolation. Many of the cells had disintegrated and the nucleus had undergone karyolysis in the fascicular zone and a few of the cells in the reticular zone were beginning to deteriorate. In the glomerular zone the cells had atrophied and the nuclei were smaller. The cells in the capsule were atrophied with an elongated nucleus. The cells in the fascicular zone of the adrenal gland from guinea pigs in group II (Fig. 14) were pale, finely vacuolated and there was a slight degeneration of the cells. The fine vacuolation was found in groups II and V, and a few cells in guinea pigs in group III were finely vacuolated. The granular cytoplasm was found extensively in the adrenal glands of guinea pigs in groups I, III, and IV. The cells in the glomerular zone and capsule of the adrenal cortex were normal in group II. In the reticular zone the cells stained darker than was found in the adrenal gland of scorbutic guinea pigs in the other groups.

There was a decrease vacuolation in adrenal glands from the animals in group III (Fig. 15). The cells in the fascicular zone were swollen and the cytoplasm of the cells appeared granular. A few of the cells of the fascicular zone were finely vacuolated. The cytoplasm in Fig. 15 is an area that is vacuolated.

Some of the vacuolations in this area are larger than in the normal guinea pigs and it appears under the microscope as a deterioration of the cytoplasm. The walls of the vacuoles are beginning to break apart. Few of the cells in the fascicular zone were normal, most of them had disintegrated. There was increased mitosis of the cells of the adrenal cortex arrested at the metaphase stage. There was also pronounced atrophy of the cells in the glomerular zone and capsule. In the fascicular and reticular zones were areas of deposits that stained a pink color. Small amounts of this pink staining material extended into the medulla.

Adrenal glands from animals in group IV (Fig. 16) contained very pale staining cells and there was a decreased vacuolation of the cells. Most of the cells of the cortex were disintegrated. The discernible cells of the fascicular zone were swollen and appeared granular. The glomerular zone and the capsule of the adrenal gland were atrophied. Pink staining deposits were also found in the fascicular and reticular zones. The entire adrenal gland contained numerous small cells that appeared as leucocytes (Fig. 17).

The adrenal glands from guinea pigs in group V (Fig. 18) were normal. There were large vacuoles and the cytoplasm contained many minute vacuoles.

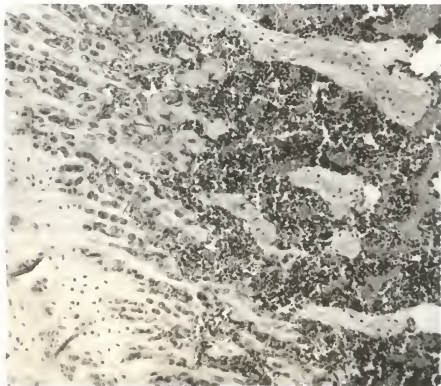


Fig. 1. Femur of a scorbutic guinea pig (group I). A distinct Trummerfeld zone is apparent at the left. There is a regression of bone matrix to an embryonic form of bone. The embryonic bone is the light staining areas. 150 X.

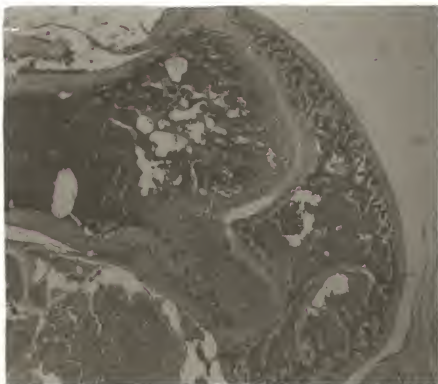


Fig. 2. Femur of a scorbutic guinea pig (group I) showing thickening of the periosteum and separation from the perichondral bone. Reversion of perichondral bone to an embryonic form of bone. There is an atrophic concave cartilage line. A marked decrease of bone matrix is noticeable. 15 X.

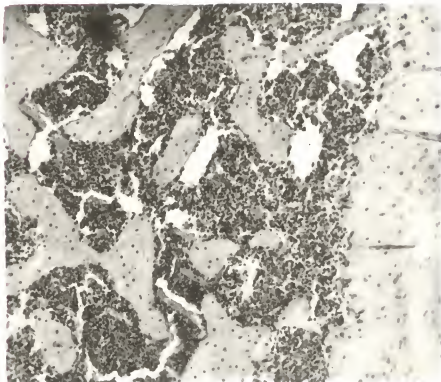


Fig. 3. Femur of a scorbutic guinea pig treated with cortisone (group II). The amount of mature bone matrix is normal, compared with the normal controls, and there is an absence of a Trummerfeld zone. 150 X.



Fig. 4. Femur of a scorbutic guinea pig treated with cortisone (group II). There is a large amount of mature bone matrix and a thick mature perichondral bone is present. No subperiosteal hemorrhage is visible. 15 X.

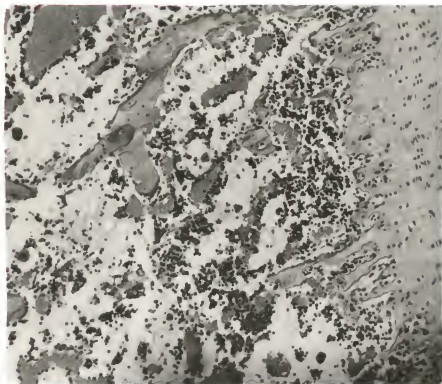


Fig. 5. Femur of a scorbutic guinea pig treated with ACTH (group III). A distinct decrease in mature bone matrix is shown. The embryonic bone stains very light. The column of cartilage cells of the Trummerfeld zone is apparent. 150 X.

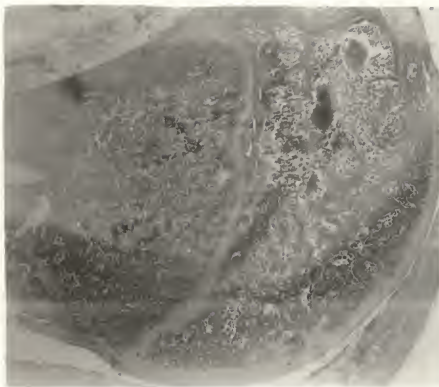


Fig. 6. Femur of a scorbutic guinea pig treated with ACTH (group III). The periosteum is greatly thickened. An embryonic form of perichondral bone is apparent. There is a decrease in mature bone matrix and a Trummerfeld zone is present. 15 X.

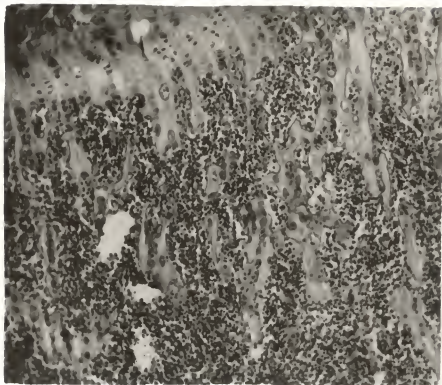


Fig. 7. Femur of a scorbutic guinea pig treated with desoxycorticosterone (group IV). A distinct Trummerfeld zone is shown. The bone matrix shows increased number of leucocytes and there is a predominate amount of embryonic bone. 150 X.

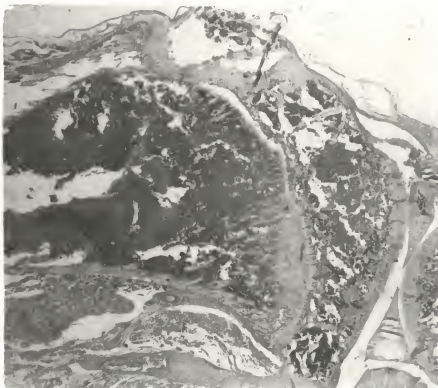


Fig. 8. Femur of a scorbutic guinea pig treated with desoxycorticosterone (group IV). The periosteum is greatly thickened and vascular. Subperiosteal hemorrhage is pronounced. There is an atrophic cartilaginous line and a distinct Trummerfeld zone. The bone matrix is composed almost entirely of embryonic bone, which is visible as very light staining material. 15 X.

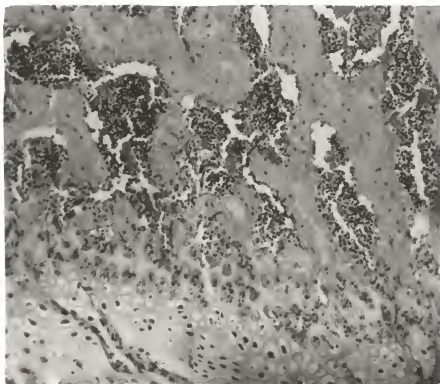


Fig. 9. Femur of a normal guinea pig (group V).
A large amount of mature bone matrix is shown.
No Trummerfeld zone is evident. 150 X.

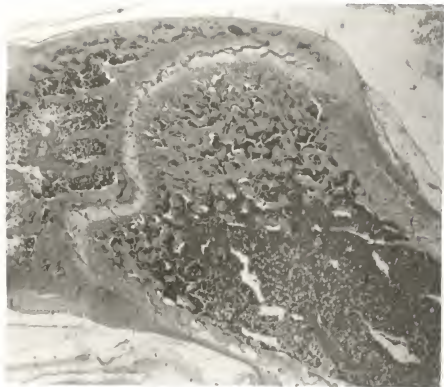


Fig. 10. Femur of a normal guinea pig (group V). There is no thickening of the periosteum and the perichondral and matrix bone are mature. 15 X.

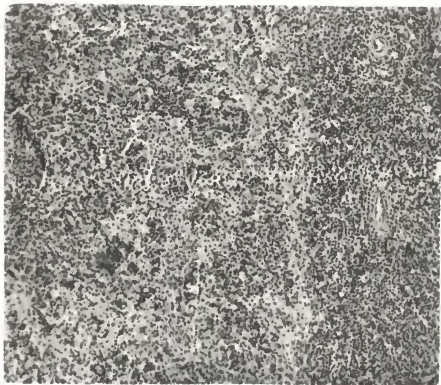


Fig. 11. Spleen from a scorbutic guinea pig treated with desoxycorticosterone (group IV). The leucocytes appear evenly distributed through the entire spleen. There is very little red pulp that can be identified. 150 X.

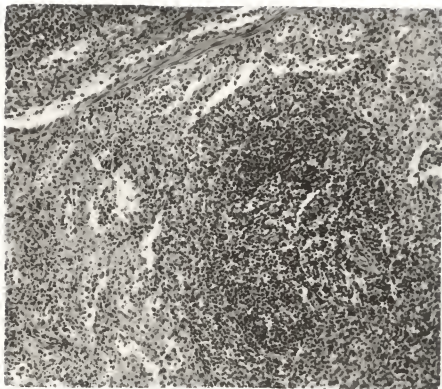


Fig. 12. Spleen from a normal guinea pig (group V). The red pulp and white pulp can be identified. Most of the leucocytes are confined to the white pulp. 150 X.

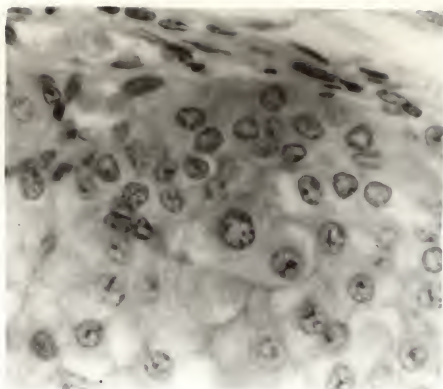


Fig. 13. Adrenal gland from a scorbutic guinea pig (group I). The cells of the capsule are atrophied with elongated nuclei. In the glomerular zone the cells are atrophied and the nuclei are smaller. The cells of the fascicular zone are hypertrophied with enlarged nuclei and the cytoplasm is granular. 1075.X.

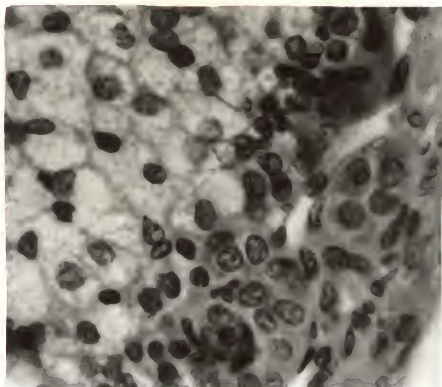


Fig. 14. Adrenal gland from a scorbutic guinea pig treated with cortisone (group II). The cells of the fascicular zone contain minute vacuoles in the cytoplasm. The glomerular zone is normal in width and the cells compare with the normal controls. 1075 X.

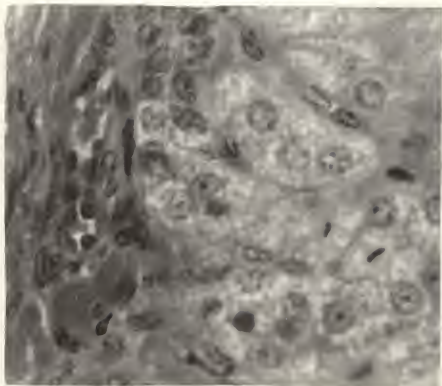


Fig. 15. Adrenal gland from a scorbutic guinea pig treated with ACTH (group III). The cells of the capsule and glomerular zones are atrophied. The cells of the fascicular zone are swollen and in this area the cytoplasm is beginning to appear granular. Some of the cells contain definite vacuoles. In the center to the right are cells undergoing mitosis. This mitosis is at the metaphase stage. 1075 X.

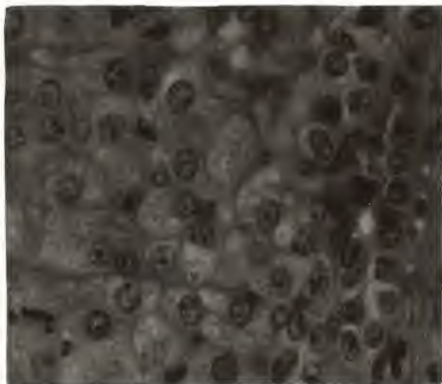


Fig. 16. Adrenal gland from a scorbutic guinea pig treated with desoxycorticosterone (group IV). The cells of the glomerular zone are swollen with enlarged nuclei. The cytoplasm is granular. The glomerular zone and capsule are atrophied. 1075 X.

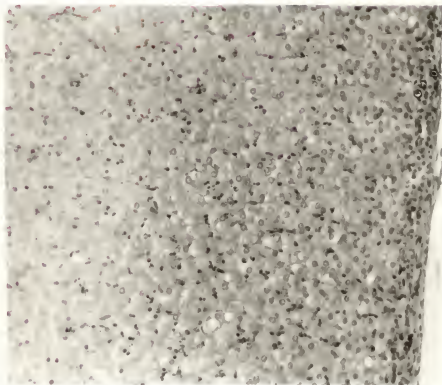


Fig. 17. Adrenal gland showing large number of cells resembling leucocytes from a scorbutic guinea pig treated with desoxycorticosterone. The cells are shown as very dark staining cells. 1075 X.

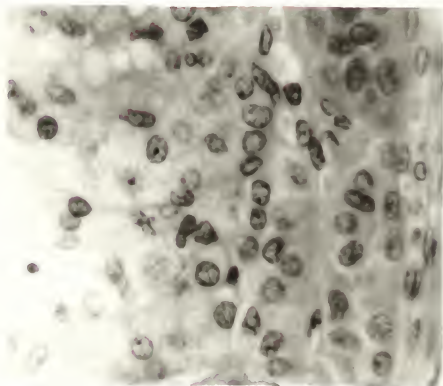


Fig. 18. Adrenal gland from a normal guinea pig (group V). There are large vacuolations and the cytoplasm also contains minute vacuoles. The cells of the glomerular zone and capsule are large and apparently normal. 1075 X.

SUMMARY AND CONCLUSIONS

1. All groups of guinea pigs showed varying degrees of scorbutic symptoms, with the exception of group V, which was normal.
2. The group of guinea pigs treated with cortisone manifested only slight scorbutic symptoms.
3. The ACTH treated guinea pigs showed more advanced symptoms of scurvy than did the guinea pigs treated with cortisone.
4. The differences in erythrocyte counts among groups were not statistically significant. There was a definite decline in erythrocytes in groups III and IV.
5. Groups of guinea pigs treated with cortisone, ACTH, or desocycorticosterone manifested a slight lymphopenia and neutrophilia as compared with the positive controls.
6. There was an eosinopenia in guinea pigs treated with cortisone and desocycorticosterone.
7. The guinea pigs treated with ACTH showed an eosinophilia as compared to the positive controls.
8. There was a decrease in packed erythrocytes and a lower hemoglobin value in guinea pigs in group IV as compared with the other groups.
9. An increased erythrocyte sedimentation rate was shown in guinea pigs treated with ACTH.
10. Guinea pigs in group I had the sharpest decline in weight and group V gained consistently. Group IV gained weight and then dropped in weight. Groups II and III were very similar to group IV.

11. Feed consumption was directly related to weight gains.

12. A highly significant difference existed in adrenal ascorbic acid between group V and the other four groups.

13. A highly significant difference existed in renal ascorbic acid between group V and the other four groups.

14. Hepatic ascorbic acid was significantly higher in group V as compared with the other four groups.

15. With the exception of the positive controls, all groups of guinea pigs showed subcutaneous hemorrhages. The guinea pigs receiving cortisone was not as severely affected as the other guinea pigs.

16. Swollen and hemorrhagic joints were found in guinea pigs in groups I, III and IV.

17. Guinea pigs in groups I, III and IV showed hemorrhage in skeletal muscle.

18. There was extreme osteoid pathology in guinea pigs in groups I and IV and severe pathological bone changes in animals in group III. The guinea pigs treated with cortisone showed very little bone pathology.

19. The ovaries of guinea pigs in group I indicated an anovulatory period. The ovaries of guinea pigs in group III and IV also indicated a period of sexual rest. Guinea pigs in groups II and V contained ovaries that showed sexual activity.

20. There were hypertrophied adrenal glands in guinea pigs in groups I, III and IV. The cells of the adrenals were swollen and many had disintegrated. Mitosis was arrested at the metaphase stage.

21. The adrenal glands of guinea pigs in group II were smaller than the normal controls.

22. Administration of desoxycorticosterone apparently aggravated the arthritic condition.

23. Injections of cortisone suppressed the clinical manifestations of scurvy.

24. From this experiment it is evident that the injections of ACTH did not stimulate the cortex of the adrenal gland to produce the hormones necessary to prevent arthritic symptoms in the scorbutic guinea pigs. This is in agreement with the theory that vitamin C may be essential in the production of the oxy-type of adreno-cortical hormones.

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HISTOPHYSIOLOGIC ANALYSES
OF SCORBUTIC GUINEA PIGS TREATED WITH
SPECIFIC ADRENO-CORTICO-ACTIVE COMPOUNDS

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ABSTRACT

Five groups of guinea pigs weighing 300 to 400 grams were fed a basal ration deficient in vitamin C. With the exception of group I, each group of guinea pigs received various injections. Group II received 5 mg. of cortisone acetate, group III received 5 units of ACTH, group IV received 5 mg. of desoxycorticosterone acetate, and group V was given 4.3 mg. of vitamin C. The hormones were injected subcutaneously at 8 hour intervals and the vitamin C was injected every 24 hours.

Blood analyses were determined and these analyses included erythrocyte count, leucocyte count, differential leucocyte count, hemoglobin concentration, erythrocyte sedimentation rate, and packed erythrocytes. Adrenal weights and adrenal ascorbic acid values were also determined.

There was a consistent decline in the total erythrocytes in group IV receiving desoxycorticosterone. In the other groups the differences in the erythrocyte counts were not significant.

There were no significant differences statistically in total leucocyte counts among the groups. There was a higher average neutrophil count for the length of the experiment in guinea pigs receiving cortisone, ACTH, and desoxycorticosterone than in the positive controls (group V) receiving vitamin C and also there was a lower lymphocyte count in these groups. But, there was not a wide enough range between groups to be statistically significant.

The average number of eosinophils for the entire experiment

was lower in groups I, II, and IV than in group V which is in agreement with most authors. The group receiving ACTH had a higher eosinophil count than group V. This is in direct contradiction to the results of some authors.

The group of guinea pigs receiving ACTH showed an increased sedimentation rate at the end of 4 hours. There were no significant differences among the other groups. Group IV manifested a consistent decrease during the experiment in packed erythrocytes. Also, the guinea pigs in group IV manifested the lowest amount of hemoglobin but it was not of statistical significance.

Guinea pigs in group I and group II manifested a sharp decline in weight from the first day of the experiment. Group III maintained its weight for the first eight days and then ensued a sharp decline. The guinea pigs in group IV gained weight for the first 12 days of the experiment, followed by a decrease in weight. The positive control animals gained weight steadily throughout the experiment. The feed consumption of the guinea pigs in each group was directly related to growth.

There was found to be a highly significant difference in adrenal ascorbic acid between group V and the other four groups. A high adrenal ascorbic acid was found in the positive control and a low content of adrenal ascorbic acid in the other four groups. The adrenal gland of group I contained the lowest amount of ascorbic acid of the five groups. Group III had slightly more adrenal ascorbic acid than group I. Adrenal ascorbic acid in group IV was slightly higher than group II; however, there was not enough difference to be of statistical value.

The liver of group V contained a high amount of vitamin C. The difference between group V and the other four groups was highly significant. There was no significant difference among groups I, II, III and IV. Group I had the lowest amount of vitamin C in the liver and group II contained more than groups III or IV.

Ascorbic acid in the kidney in group V was also higher than in the other four groups and there was very little difference among the first four groups in ascorbic acid content of the kidney.

The adrenal glands in groups I, III, and IV were larger than the normal controls and the adrenal glands from group II were smaller than the normal controls.

With the exception of the positive controls, all groups of guinea pigs showed subcutaneous hemorrhage. The guinea pigs receiving cortisone were not as severely affected as the guinea pigs in the other groups.

Guinea pigs in groups I, III, and IV showed hemorrhage in skeletal muscle. These same guinea pigs manifested swollen and hemorrhagic joints.

The organs that showed the most histopathology were the bone, ovaries, and adrenal glands. There was extreme osteoid pathology in guinea pigs in groups I and IV and less severe pathological bone changes in animals in group III. The guinea pigs treated with cortisone showed very little bone pathology. The most noticeable pathology was a reversion of mature bone to an embryonic form of bone.

The ovaries of guinea pigs in group I indicated an anovulatory

period. The ovaries of guinea pigs in group III and IV also indicated a period of sexual rest because no corpora lutea were present; but, the ovaries did contain large follicles. Guinea pigs in groups II and V possessed ovaries that contained both corpora lutea and large follicles.

There were hypertrophied adrenal glands in guinea pigs in groups I, III, and IV. The cells of the adrenals were swollen and many had disintegrated. Increased mitosis was shown and it was arrested at the metaphase stage. The adrenal glands of guinea pigs in group II were smaller than the normal controls. The cells of the fascicular zone of the adrenal cortex stained darker compared with the normal controls.

All groups of guinea pigs showed varying degrees of scorbutic symptoms, with the exception of group V. The group of guinea pigs treated with cortisone manifested only slight scorbutic symptoms. The ACTH treated guinea pigs showed more advanced symptoms of scurvy than did the guinea pigs treated with cortisone.

Administration of desoxycorticosterone apparently aggravated the arthritic condition, and, conversely, cortisone suppressed these clinical manifestations, which is in agreement with previous work. From this experiment it is evident that the injections of ACTH did not stimulate the cortex of the adrenal gland to produce the hormones necessary to prevent arthritic symptoms in the scorbutic guinea pigs. This is in agreement with the theory that vitamin C may be essential in the production of the oxy-type of adreno-cortical hormones.

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